REVASCULARIZATION OF IMMATURE AND MATURE PERMANENT TEETH WITH NECROTIC PULP AND OPEN APICES : AN IN VIVO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"REVASCULARIZATION OF IMMATURE AND MATURE PERMANENT TEETH WITH NECROTIC PULP AND OPEN APICES : AN IN VIVO STUDY"** is a bonafide record of work done by **Dr. SN. PADMARAJ** under our guidance during the study period **2010 – 2013.**

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF RADIOGRAPHIC IMAGE

ABSTRACT

BACKGROUND :Possibility of tissue regeneration within the pulp space and continued root development in teeth with necrotic pulps and open apices.

AIM: The aim of this in vivo study was to evaluate the revascularization procedure both in immature and mature permanent teeth with necrotic pulp and open apices, disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

METHODOLOGY : 15 patients were selected who presented with immature and mature permanent teeth with pulpal necrosis and open apices. In the first visit, the root canal were accessed with LA and rubber dam isolation, then the canal was disinfected using triple antibiotic paste containing ciprofloxacin, metronidazole and clindamycin in the ratio of1:1:1 and closed with IRM . In the second visit, after administering local anaesthesia and isolating with rubber dam, triple antibiotic paste was washed out by saline irrigation and apical papilla beyond the confines of the root canal were stimulated with sterile H file to produce a blood clot. Finally the access was closed using a double seal with MTA placed apical to cementoenamel junction and resin bonded cement over the MTA. Radiographic examination and pulp sensibility test was done during the follow up period of 2,4,6,8 and 10 months.

RESULTS :

The results showed that, all the patients who reported till the 10 months follow up showed complete resolution of periapical radiolucencies. 10 Out of 13 patients, who reported with a period of 10 months follow up showed root development and apical closure. 8 out of 13 patients who reported with a period 10 months follow up showed root development, apical closure and lateral thickening of radicular dentin.2 out of 13 patients at the end of 10 month follow up showed positive response to electric pulp test

CONCLUSION:

Within limitation of this study, it can be concluded that there is evidence of root development, increase in lateral wall thickness, apical closure and positive response to pulp sensibility test were observed in both mature and immature teeth with necrotic pulp.

Keywords:

Revascularization, triple antibiotic paste, MTA, blood clot

INTRODUCTION

Endodontic management of immature permanent teeth with necrotic pulps and open apices pose a challenge to a dentist. This is due to the presence of thin dentin walls and the lack of a natural apical constriction against which the obturation material is placed.[8] Open apex can occur as a result of pulpal necrosis due to caries or trauma, before the completion of root development. It can also form in a mature root as a result of extensive apical resorption due to orthodontic treatment, inflammatory root resorption and periradicular surgery.[39]

The traditional management of the open apex was by customized gutta-percha cone method with or without apical surgery and retrograde filling procedures. The potential drawback of surgical approach is, it can damage the very thin dentinal wall of the open apex tooth. Unfavorable crown root ratio is another reason of concern. Both these causes, lead to complication after post endodontic restoration (viz crown and root fracture).[29]

For the past five decades apexification has been the treatment of choice in teeth with open apex which was introduced by Kaiser in

1964.[31] This technique basically induces the formation of apical barrier at the apex by placement of calcium hydroxide. But the main drawback of apexification is the duration of treatment period (multiple visits). The increased susceptibility of the root to fracture after calcium hydroxide exposure and varying degree of apical closure it exhibits, are other potential disadvantages.[39]

The evolution of MTA as an artificial apical barrier material has drastically changed the conventional means of managing an open apex tooth. There is no denying in the fact that MTA induces a barrier in open apex cases. It does not induce the thickening of the radicular dentin. In this scenario the role of contemporary techniques in the management of open apex is highly questionable.[39]

The emergence of regenerative endodontics as a contemporary therapeutic modality has entirely altered the conventional approach for management of open apex. The main role of regenerative endodontics is to give life to a bioengineered tooth. It can drastically alter the compromised structural integrity of diseased tooth in a positive way.[39]

Revascularization is a regenerative endodontics procedure, since the blood clot is a rich source of growth factor, it is always possible that

it can lead to differentiation, growth, and maturation of fibroblast, odontoblast, and cementoblast. It was Ostby in 1961 who introduced the concept of revascularization, which was followed by Rule and Winter. Rule and Winter showed that by revascularization, root development and apical barrier formation can occur in tooth with necrotized pulp. Literature survey clearly shows this treatment modality of revascularization can result in radiographically visible normal matured tooth which had previously immature open apex.[51]

It is a well established fact that development of root takes place from Hertwig's epithelial root sheath. As the root develops the HERS disintegrates and disappears but not completely. The remnants of HERS are normally found in the open apex of the developed root which is highly resistant to polymicrobial infection and pulpal necrosis. As the spreading of infection in pulp is per tissue compartment, there is always a possibility that the apical pulpal tissue can remain vital. It is now being argued, this vital apical pulpal tissue with the remnants of HERS can contribute to continued root development and lengthening, even after the establishment of necrosis.[29]

The other two mechanisms which are being put forth for continued root development are the role played by stem cell of apical papilla (SCAP) and multipotent pulp stem cells which are seen abundance in immature tooth and which have the capacity to lay down bone and cementum.[17]

The infected root canal system is a sanctuary for a plethora of microorganisms both aerobic and anaerobic. Elimination of these microorganisms are the prime goal for modern endodontic therapy. Due to varying degree of sensitivity exhibited by microorganism towards the different antibiotics and due to the ability of microorganism to rapidly exhibit resistant to antibiotics, a combined antibiotic regimen has been advised. The recommended regimen includes metronidazole, ciprofloxacin and minocycline. An alternate antibiotic regimen includes clindamycin instead of minocycline due to the potential discoloring ability of minocycline.[42]

Literature is replete with documented case report of revascularization of necrotic root canal by inducing bleeding into the canal by over instrumentation. The two main advantages of revascularization by induced bleeding are, firstly, it is less technique

sensitive. Secondly, the possibility of immune rejection and pathogen transmission are very minimal since regeneration occurs by patient's own biological tissue.

AIM:

The purpose of this in-vivo study was to evaluate the revascularization procedure both in immature and mature permanent teeth with necrotic pulp and open apices, disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

OBJECTIVE:

The objective of this study was,

1. to assess the root development, thickening of lateral walls, and apical closure by radiograph
2. to assess the pulp sensibility by electric pulp tester.

REVIEW OF LITERATURE

Kling et al (1986)[32] conducted a study to determine the frequency of pulp revascularization in therapeutically reimplanted incisors as well as its relationship with the following factors: width of apical foramen, duration of extra-alveolar time, storage conditions and postoperative administration of antibiotics. Thus concluded that all teeth in which revascularizalion did not occur exhibited a periapical radiolucency and/or external inflammatory root resorption The advantages of pulp revascularization lie in the possibility of further root development and reinforcement of dentinal walls by deposition of hard tissue, which is lost by endodontic treatment.

Trope YK (2000)[63] et al conducted a study to determine the effect of topical treatment with doxycycline and/or the application of unfilled resin to the anatomical crown on the occurrence of revascularization in reimplanted dog teeth. This study confirmed the clinical benefit of doxycycline in enhancing revascularization of replanted dog teeth. However, more research should be done in order to evaluate the pathways of bacterial penetration after traumatic injuries and also to find the treatment method to eliminate this

penetration. Presumably the longer the pulp space can be kept free of bacteria, the higher will be the incidence of revascularization of these teeth.

Iwaya et al (2001)[27] conducted a case series wherein – A necrotic immature mandibular second premolar with periapical involvement in a 13-year-old patient was treated. Instead of the standard root canal treatment protocol and apexification, antimicrobial agents were used in the canal, after which the canal was left empty. Radiographic examination showed the start of apical closure 5 months after the completion of the antimicrobial protocol. Thickening of the canal wall and complete apical closure was confirmed 30 months after the treatment, indicating the re-vascularization potential of a young permanent tooth pulp into a bacteria-free root canal space.

Giuliani V (2002)[18] reported a case series in which three clinical cases have been treated with the use of an apical plug of MTA for apexification. All three cases were central incisors that had suffered premature interruption of root development as a consequence of trauma. According to the treatment protocol, the root canals were rinsed with 5%NaOCl; then, calcium hydroxide paste was placed in

the canals for 1week. Consequently, the apical portion of the canal (4mm) was filled with MTA. The remaining portion of the root canals was then closed with thermoplastic gutta-percha. At 6-month and 1-year follow-up period the clinical and radiographic appearance of the teeth showed resolution of the periapical lesions. MTA appears to be a valid option for apexification with its main advantage being the speed at which the treatment can be completed.

Banchs & Trope (2004)[6] presented a new technique to revascularize immature permanent teeth with apical periodontitis. The canal is disinfected with copious irrigation and a combination of three antibiotics. After the disinfection protocol is complete, the apex is mechanically irritated to initiate bleeding into the canal to produce a blood clot to the level of the cementoenamel junction. The double seal of the coronal access is then made. Finally, they concluded that the combination of a disinfected canal, a matrix into which new tissue could grow, and an effective coronal seal appears to have produced the environment necessary for successful revascularization.

Windley W et al (2005)[61] conducted a study to assess the efficacy of a triple antibiotic paste in the disinfection of immature dog teeth with apical periodontitis. The canals were sampled before (S1)

and after (S2) irrigation with 1.25% NaOCl and after dressing with a triple antibiotic paste (S3), consisting of metronidazole, ciprofloxacin, and minocycline. The results indicate the effectiveness of a triple antibiotic paste in the disinfection of immature teeth with apical periodontitis.

Petrino JA (2007)[47] presented a case series on revascularization procedure as described by Banchs and Trope. Six immature teeth with apical periodontitis (in three patients) were treated via the revascularization protocol using irrigants, a triple antibiotic paste, and a coronal seal of mineral trioxide aggregate and composite. For follow-up, all six teeth showed resolution of periapical radiolucencies, whereas three of six teeth showed continued root development. Two teeth displayed a positive response to vitality testing.

Murray PE (2007)[43] reviewed regenerative endodontics and its goals, and describes possible techniques that will allow regenerative endodontics to become a reality. These potential approaches include root-canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds and gene therapy and concluded that

that regenerative endodontics is an inevitable therapy, and they call for action from scientists, funding agencies, and the endodontic profession to pool resources to hasten its development. The unleashed potential of regenerative endodontics may benefit millions of patients each year.

Doyon GE et al (2006)[15] conducted a study to determine if exposure to intracanal calcium hydroxide [Ca(OH2)] alters the fracture resistance of human root dentin. One hundred and two freshly extracted single rooted human teeth divided into three groups of 34 teeth each. Coronal access and endodontic instrumentation using round burs, stainless steel files, and Profile GT rotary files were completed for each tooth. The prepared root canal system of each tooth was filled with saline solution (group 1), USP Ca(OH)2 (group 2), or Metapaste (group 3). The apices and access openings were sealed with composite resin and the teeth were immersed in saline. After 30 days, the roots of 17 teeth from each group were sectioned horizontally into 1mm thick disks and each disk was loaded to fracture at 2.5 mm/min with a SATEC universal-testing machine. After 180 days the same procedure was performed on the remaining 17 teeth in each of the 3 groups. The peak load at fracture was

measured for each dentin disk. After 30 days exposure to the test solution, there was no difference in the peak load at fracture for the three groups of teeth. However, after 180 days, the roots of the teeth exposed to USP Ca(OH)2 showed a significant decrease in peak load at fracture when compared to the 30-day groups and the 180-day groups exposed to saline or Metapaste.

Witherspoon DE (2008)[62] conducted a retrospective analysis of the outcome of initial nonsurgical root canal treatment of teeth with open apices, obturated with mineral trioxide aggregate when no apical barrier existed and concluded that MTA obturation of canals with open apices is a viable alternative to the use of calcium hydroxide to induce apical closure.

Trope m (2008)[59] conducted a review wherein he stated that the regenerative potential of dental pulp, particularly in mature teeth, has been considered extremely limited. However, our improved understanding of pulpal inflammation and repair and improved dental materials and technologies make vital pulp therapy a viable alternative to root canal treatment. This article explores our knowledge in this regard and the future potential of saving or even regenerating the pulp as a routine dental procedure.

Holden DT (2008)[23] conducted a retrospective study to evaluate the clinical outcomes of ProRoot mineral trioxide aggregate used as an artificial apical barrier in teeth with immature apices. Twenty teeth from 19 patients were included in this study. A healed diagnosis was based on periapical index scores of 1 or 2 and no clinical signs or symptoms at recall examinations Overall, these results indicated that the mineral trioxide aggregate apical barrier technique is a successful method for obturating teeth with immature apices.

Sonoyama w et al (2008)[54] did a pilot study on the apical papilla of stem cell (APSC) and concluded that the apical papilla harbors multipotent MSCs that express various MSC markers. They are capable of forming odontoblast like cells, produce dentin in vivo, and are likely to be the cell source of primary odontoblasts for the root dentin formation.

Huang GTJ (2008)[24] reviewed the new concept of regenerative endodontics in the management of immature permanent teeth. A new protocol of treating endodontically involved immature permanent teeth based on published articles to date was summarized in the review. The key procedures of the protocol are (1) minimal or

no instrumentation of the canal while relying on a gentle but thorough irrigation of the canal system, (2) the disinfection is augmented with intra-canal medication of a triple-antibiotic paste between appointments, and (3) the treated tooth is sealed with mineral trioxide aggregate (MTA) and glass ionomer/resin cement at the completion of the treatment.

Cotti E et al (2008)[11] reported a case in which he described the treatment of a necrotic immature permanent central incisor with complete crown fracture, suspected root fracture, and sinus tract, which was not treated with conventional apexification techniques. The root canal was gently debrided of necrotic tissue with a sharp spoon excavator and irrigated for only one third of its length with NaOCl and then medicated with calcium hydroxide. After 15 days the sinus tract had healed, and the tooth was asymptomatic. The tooth was accessed, calcium hydroxide was removed, bleeding was stimulated to form an intracanal blood clot, and mineral trioxide aggregate was placed coronally to the blood clot. After 8 months, a coronal calcified barrier was radiographically evident and accompanied with progressive thickening of the root wall and apical

closure. Two and a half years after treatment was initiated, the tooth remained asymptomatic, and the sinus tract had not reappeared.

Young Jung et al (2008)[30] in his case series reported the outcomes of 8 patients (ages 9 –14 years) who presented with 9 immature permanent teeth with pulpal necrosis and apical periodontitis After NaOCl irrigation and medication with ciprofloxacin, metronidazole, and minocycline, these teeth were sealed with mineral trioxide aggregate and restored. The other group of 4 teeth had no evidence of any residual vital pulp tissue. This second group of teeth was treated with NaOCl irrigation and medicated with ciprofloxacin, metronidazole, and minocycline followed by a revascularization procedure. In both groups of patients, there was evidence of satisfactory post-operative clinical outcomes (1–5 years); the patients were asymptomatic, no sinus tracts were evident, apical periodontitis was resolved and there was radiographic evidence of continuing thickness of dentinal walls, apical closure, or increased root length.

Shah N (2008)[51] conducted a pilot study is to evaluate the efficacy of revascularization in 14 cases of infected, immature teeth. Endodontic treatment was initiated and after infection control,

revascularization was performed. The access cavity was sealed with glass ionomer cement. The cases were followed up at regular intervals of 3 months. Then the outcomes are radiographic resolution of periradicular radiolucencies was judged to be good to excellent in 93% (13 of 14) of the cases. In the majority of cases, a narrowing of the wide apical opening was evident. In 3 cases, thickening of apical dentinal walls and increased root length were observed. The striking finding was complete resolution of clinical signs and symptoms and appreciable healing of periapical lesions in 78% (11 of 14) of cases. Thickening of lateral dentinal walls was evident in 57% (8/14) of cases, and increased root length was observed in 71% (10/14) of cases. None of the cases presented with pain, reinfection, or radiographic enlargement of preexisting apical pathology. This pilot study documented a favourable outcome of revascularization procedures conducted in immature nonvital, infected permanent teeth.

Thibodeau et al in (2008)[55] reported a case wherein revascularization of the necrotic infected pulp space of an immature permanent maxillary central incisor tooth was induced in vivo by stimulation of a blood clot from the periapical tissues into the canal space. This was achieved after disinfection of the canal space with a

topical antibiotic paste. They reported that, this treatment approach offers clinicians, great potential to avoid the need for traditional apexification with calcium hydroxide or the need to achieve an artificial apical barrier with MTA. Furthermore, this treatment approach can help rescue infected immature teeth by physiologically strengthening the root walls

Raldi DP et al (2009)[49] conducted a clinical study in which three clinical cases involving teeth with open apices and apical periodontitis were treated using different protocols. The first case was managed with intracanal calcium hydroxide paste for 12 months before obturation with gutta-percha and sealer. In the second case, an apical plug of mineral trioxide aggregate (MTA) was used before obturation with gutta-percha and sealer and treatment was completed during 2 appointments. In the third case, the tooth, which had a divergent root canal system, was completely obturated with MTA and treatment was also completed over 2 appointments. In all 3 cases, signs of bone healing were observed after treatment.

Yilmaz S et al (2009)[64] conducted a study to assess the healing response of intra bony defects following regenerative treatment with platelet-rich plasma (PRP) combined with a bovine-derived xenograft

(BDX) in smokers and non-smokers. A total of 24 advanced chronic periodontitis patients, 12 smokers and 12 non-smokers, with 113 intra bony defects with an intra bony component ofX3mm were included in this study. Defects were surgically treated with PRP/BDX. At baseline and 12 months after surgery, the following parameters were recorded: plaque and sulcus bleeding indices, probing depth (PD), relative attachment level, marginal recession, probing and radiographic bone levels concluded that within the limits of this study, the results indicate that treatment outcome following PRP/BDX application in intra bony defects is impaired with smoking.

Nakamura S (2009)[45] et al conducted a study focused on the characterization of stem cells from human exfoliated deciduous teeth (SHED) compared with dental pulp stem cells (DPSCs) and bone marrow–derived mesenchymal stem cells (BMMSCs) in which he have compared ‘‘stemness’’ such as the proliferation rate and the expression of stem cell marker of DPSCs, SHED, and BMMSCs. In addition, gene expression profile of DPSCs and SHED were analyzed by using DNA microassay and concluded that the pathways and individual genes identified in this study might support SHED as an

alternative useful cell source for future clinical applications containing novel therapy for systemic diseases.

Mohammadi Z (2009)[42] reviewed the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology and concluded that Clindamycin and a combination of three antibiotics (metronidazole, ciprofloxacin and minocycline) have also been reported to be effective at reducing bacterial numbers in the root canal systems of infected teeth.

Huang A (2009)[26] conducted a study to raise a novel idea to source hDPSCs from complicated crown-fractured teeth requiring root canal therapy. hDPSCs were harvested from the pulp tissues for two complicated crown fractured teeth requiring root canal therapy, retaining the teeth for subsequent prosthodontic rehabilitation and finally concluded that pulp exposed in complicated crown-fractured teeth might represent a valuable source of personal hDPSCs.

Mohammadi Z (2009)[41] in his article reviewed that antibiotics are an extremely valuable addition to the armamentarium available to health practitioners for management of bacterial infections. Due to the potential risk of adverse systemic effects of systemic applications

and ineffectiveness of systemic antibiotics in the necrotic pulpless tooth and the periradicular tissues, local application of antibiotics may be a more effective mode for delivering antibiotics to infected root canals. This paper reviews the history, rationale, and applications of antibiotics and antibiotics containing medicaments in endodontics.

Huang GTJ (2009)[25] overviewed the recent concept of pulp revitalization in the treatment of immature teeth with nonvital pulps and the emerging research on pulp tissue engineering and regeneration and concluded that there is some concern caused by the uncertainty as to how pulp regeneration would affect the future of endodontic practice. One may anticipate that to feasibly deliver stem cell-based endodontic therapy for pulp/dentine regeneration in endodontic practice, an uncomplicated clinical protocol would need to be established.

Bose R et al (2009)[5]conducted a retrospective study in which several case reports on endodontic regeneration involving immature permanent teeth have recently been published. These case series have used varying treatments to achieve endodontic regeneration including triple antibiotic paste, Ca(OH)2 and formocresol. However, no study has analyzed the overall results.

Ding et al (2009)[14] in their clinical study recruited twelve patients, each with an immature permanent tooth with chronic or acute apical periodontitis. A triantibiotic mix (ciprofloxacin, metronidazole, and minocycline) was used to disinfect the pulp for 1 week and then a blood clot was created in the canal, over which grey mineral trioxide aggregate was placed. At the end of 18 month follow up, the teeth were found to exhibit complete root development, with a positive response to pulp testing. Finally they concluded that revascularization procedure could be effective for managing immature permanent teeth with apical periodontitis with appropriate case selection.

Chueh LH, et al (2009)[10] conducted a retrospective study included 23 necrotic immature permanent teeth treated for either short-term or long-term using conservative endodontic procedures with 2.5% NaOCl irrigations without instrumentation but with $Ca(OH)_2$ paste medication. For seven teeth treated short-term, the gutta-percha points were filled onto an artificial barrier of mineral trioxide aggregate (MTA). For 16 teeth treated long-term, the gutta-percha points, amalgam, or MTA were filled onto the $Ca(OH)_2$-induced hard tissue barrier in the root canal and concluded that

immature permanent teeth with pulp necrosis and apical pathosis can still achieve continued root development after proper short-term or long-term regenerative endodontic treatment procedures.

Mathew BP(2010)[43] in his case series reported that the golden rule in the practice of endodontology, is to debride and obturate the canals as efficiently and three dimensionally as possible in an amount of time and appointments that are reasonable to the patient. Whilst most endodontic cases can be managed predictably and comfortably, there are a group of patients that defy predictable routine treatment. This group is composed of those who present with immature apical formation. Such teeth with incomplete rhizogenesis, pose a special challenge to dentists all over because of large open apices , divergent root walls, thin dentinal walls that are susceptible to fracture and frequent periapical lesions. Patients who present with non-vital immature teeth pose a special challenge to dentists and requires a specially tailored treatment plan. This paper presents a brief review of the causes and complications of incomplete root development and the various treatment options reported in the literature with respect to their management with case reports.

Nagaveni NB (2010)[44] conducted a study to report the successful closure of root apex in pulpless permanent incisors with wide open apices in two pediatric patients using single CaOH dressing. Endodontic management of immature non vital permanent teeth in young pediatric patients is a great challenge to dentists. The walls of the root canals are frequently divergent and open apexes make debridement and obturation difficult. Thus closure of root apex is very essential for success of the endodontic treatment. Although different materials are used for the apexification procedure, calcium hydroxide is the material of choice for apical barrier formation and healing. There are different opinions regarding frequency of CaOH dressing change to induce complete closure of the apex. Literature suggests that dressing should be changed frequently.

Petrino JA (2010)[46] reported a case series in wherein three cases that used revascularization protocol as described by Banchs and Trope. Each case presented its own special circumstances and challenges. In this study six immature teeth with apical periodontitis (in three patients) were treated via the revascularization protocol using irrigants, a triple antibiotic paste, and a coronal seal of mineral trioxide aggregate and composite. Results from this case series

showed that revascularization is a technically challenging but effective treatment modality for the immature tooth with apical periodontitis.

GURTU (2010)[21] reported in his case series that The term 'open apex' is used to indicate the presence of an exceptionally wide root canal at the apex. Open apices occurs in immature teeth when root development ceases as a result of pulp necrosis. Trauma and caries are regarded as the main cause of open apices in immature anterior teeth. Successful root canal treatment occurs when over instrumentation and overfilling are avoided and filling materials confined to the limits of the canal. Hence, accurate working length is essential for optimal healing. Open apices pose many difficulties to contemporary methods of canal length determination. A case report presents a consistent tactile method for working length determination in teeth with open apices.

Godoyl FG et al (2011)[19] did a review on the recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth and concluded that the traumatized tooth must be non-vital and not be suitable for apexogenesis, apexification, partial pulpotomy, or root canal

obturation treatments. The tooth must be permanent and very immature with a wide-open apex and exposed pulp. The tooth must have thin walls that will benefit from a continued development of the root, so that it can become stronger and less prone to failure in later life. The patient must be aged 7–16 years, in good health, and have parents/guardians willing to take them to attend multiple appointments.

Kubasad GC (2011)[34] reported a case review wherein successful healing and apexification using MTA was reported. The case reports present two cases with traumatized upper anterior teeth. The radiographic evaluation revealed open apices with blunderbuss canals, the canal was cleaned using intracanal instruments and 5.25% NaOCl and final irrigation with 2% chlorohexidine in subsequent appointments 3-4mm apical stop was created with mineral trioxide aggregate and allowed to set. Subsequently the root canals were obturated with thermoplasticized gutta-percha. And concluded that apexification in one step using an apical plug of MTA can be considered a predictable treatment and may be an alternative to use a long term calcium hydroxide apexification.

Iwaya S I et al (2011)[28] reported a case in which an immature permanent mandibular central incisor with periapical involvement in a 7-year-old boy was treated to promote revascularization. The root canal was not mechanically cleaned during the treatment period, but was irrigated with hydrogen peroxide and sodium hypochlorite. Calcium hydroxide compound was used for disinfection. At the fifth visit vital tissue appeared in the canal near the apical region, and calcium hydroxide compound was placed in contact with the soft tissue in the root canal. The access cavity was sealed with glass–ionomer cement followed by an adhesive composite resin filling. Radiographic examination 30 months after the initial treatment confirmed closure of the apex and thickening of the root wall. The case was observed for up to 13 years and root development was confirmed.

Cehreli Z C et al (2011)[8] presented a case series demonstrated the outcome of revascularization treatment with intracanal medicament of calcium hydroxide in immature necrotic molars. In this study immature necrotic permanent first molars of patients 8–11 years old were treated by a revascularization protocol that used 2.5% NaOCl irrigation, medication with calcium hydroxide placed in the

coronal third of the root canals, induction of apical bleeding, and coronal sealing with white mineral trioxide aggregate. On the basis of a follow-up period of 10 months, the present cases demonstrated a favorable outcome of the revascularization procedure in immature necrotic molars by using calcium hydroxide medication in the coronal third of the root canals.

Buch A (2011)[7] reviewed the management of open apex where in he stated that golden rule in endodontics is to debride and obturate the canals as efficiently and three dimensionally as possible in reasonable time and appointments. Open apices of pulpless immature teeth pose a major challenge in this regard. This article includes open apex cases treated with one step apical barrier technique using Mineral Trioxide Aggregate and with Calcium Hydroxide apexification and concluded that Considering the time factor and the predictability of a three dimensional seal, MTA proves to be a better option for managing patients with open apices. However whether to select MTA or calcium hydroxide depends upon cost and number of appointments patient can afford. Commercial products made out of MTA are still expensive, thus making use of MTA unaffordable for a number of patients.4 Thus, selecting the correct technique and its

proper implementation helps an endodontist to successfully overcome the so-called "dilemma of open apex!"

Torabinejad M and Turman. M (2011)[57] presented a case report in which maxillary second premolar tooth had been accidently extracted and immediately replanted developed pulpal necrosis and symptomatic apical periodontitis. After preparing an access cavity, its necrotic pulp was removed. The canal was irrigated with 5.25% NaOCl solution and dried with paper points. A triple antibiotic mixed with distilled water was packed in the canal and left for 22 days. Twenty milliliters of whole blood was drawn from the patient's forearm for preparation of PRP. After removal of the antibiotic mixture, the PRP was injected into the canal space up to the cementoenamel junction level. Three millimeters of grey mineral trioxide aggregate was placed directly over the PRP clot. Three days later, the tooth was double-sealed with permanent filling materials and concluded that regeneration of vital tissues in a tooth with necrotic pulp and a periapical lesion is possible; PRP is potentially an ideal scaffold for this procedure.

Tyler W. Lovelace, Michael A. Henry(2011)[38] through molecular analysis found significant accumulation of transcripts for

the stem cell markers CD73 and CD105 (up to 600-fold) in the blood sample collected from the root canal systems(after inducing bleeding from the periapex), compared with levels found in the systemic blood. Histologic analysis demonstrated that the delivered cells expressed both CD105 and STRO-1, markers for a subpopulation of mesenchymal stem cells. Collectively, these findings demonstrate that the evoked bleeding step in regenerative procedures triggers the significant accumulation of undifferentiated stem cells into the canal space where these cells might contribute to the regeneration of pulpal tissues seen after antibiotic paste therapy of the immature tooth with pulpal necrosis. periodontitis/abscess to revascularization procedures.

Demarco et al.(2011)[13] reviewed the most recent endeavors to regenerate pulp tissue based on tissue engineering principles and provides insightful information to readers about the different aspects involved in tissue engineering. They speculate that the search for the ideal combination of cells, scaffolds, and morphogenic factors for dental pulp tissue engineering may be extended over future years and result in significant advances in other areas of dental and craniofacial research. The findings collected in this literature review show that we are now at a stage in which engineering a complex tissue, such as the

dental pulp, is no longer an unachievable goal and the next decade will certainly be an exciting time for dental and craniofacial research.

Lenzi R (2012)[35] presented a case report in which the revitalization of a previously necrotic pulp space has been shown to be possible and even considered predictable. 2 teeth were treated according to priniciples laid by Banch and Trope. One of the teeth failed to revitalized, whereas the other which was expected to succeed revitalization. In the tooth that failed to revitalize, auto-apexification occurred and concluded that complete understanding of the criteria for predictable revitalization and apexification is still lacking.

Torabeinejad et al (2012)[56] reported in his case wherein he evaluated the clinical, radiographic and histological findings of a regenerative procedure using platelet-rich plasma (PRP). On examination of the tissue removed from the root canal of this tooth revealed the presence of a vital pulp-like vital connective tissue. Based on these findings, it appears that pulp-like tissue can be generated in a human tooth with the use of PRP as a scaffold in regenerative endodontic procedures.

Chen MY, Chen KL (2012)[9] observed five types of responses of the immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Type1, increased thickening of the canal walls and continued root maturation; Type 2, no significant continuation of root development with the root apex becoming blunt and closed; Type 3, continued root development with the apical foramen remaining open; Type 4, severe calcification (obliteration) of the canal space; Type 5, a hard tissue barrier formed in the canal between the coronal MTA plug and the root apex. Based on the results obtained from their case series they concluded that the outcome of continued root development was not as predictable as increased thickening of the canal walls in human immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess after revascularization procedures.

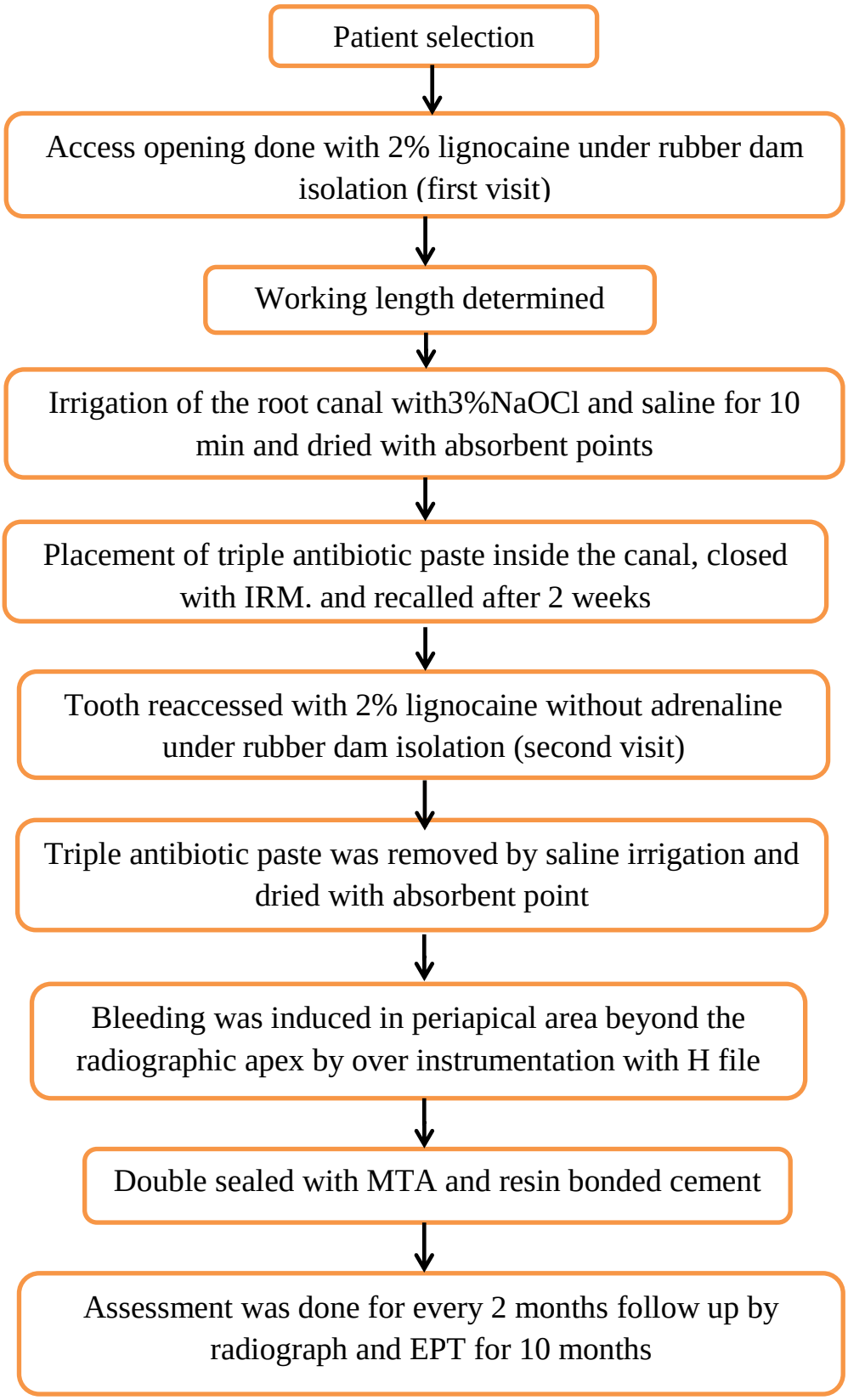
Shimizu E et al (2012)[52] reported a case series wherein he reported that histological studies of immature human permanent necrotic teeth with or without apical periodontitis after revascularization have not been reported. This case report describes the histological findings of tissue formed in the canal space of an immature permanent tooth #9 with irreversible pulpitis without apical

periodontitis after revascularization. An immature human permanent tooth #9 was fractured 3.5 weeks after revascularization and could not be retained. The tooth was extracted and prepared for routine histological and immunohistochemical evaluation in order to examine the nature of tissue formed in the root canal following the revascularization procedure. At 3.5 weeks after revascularization, more than one half of the canal was filled with loose connective tissue similar to the pulp tissue. A layer of flattened odontoblast-like cells lined along the predentin. Layers of epithelial-like cells, similar to the Hertwig's epithelial root sheath, surrounded the root apex. No hard tissue was formed in the canal and concluded that based on the histological findings in the present case, regeneration of pulp-like tissue is possible after revascularization. In this case, both the apical papilla and the Hertwig's epithelial root sheath survived in an immature permanent tooth despite irreversible pulpitis but without apical periodontitis.

Ruparel et al (2012)[50] conducted a study in the direct effect of intracanal medicaments on survival of stem cells of the apical papilla. In which SCAPs were cultured and subjected to either no drug treatment or various concentrations including TAP, DAP, modified

TAP. Viable stem cells counts were obtained using an automated method of detecting tryphan blue dye at 3 days after treatment. All 4 antibiotics significantly reduced SCAP survival in a concentration dependent fashion. Interestingly, Ca(OH)2 was conducive with SCAP survival at all concentrations. And finally concluded that Intracanal medicaments must be used at concentrations that are bactericidal while having minimal effects on stem cell viability.

Procedural sequence for pulp revascularization



MATERIALS AND ARMAMENTARIUM(PHOTOGRAPH)

Fig: 1 Materials

Fig 2: Clinical Armamentarium

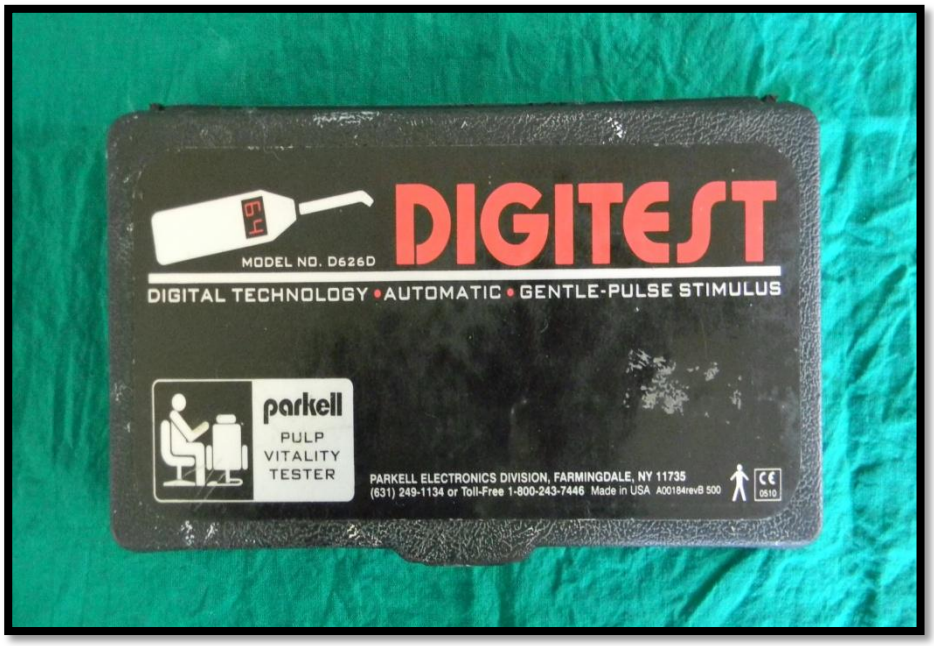


Fig 3: Electric Pulp Tester

Fig 4: Light Curing Unit

PHOTOGRAPH

CLINICAL CASE

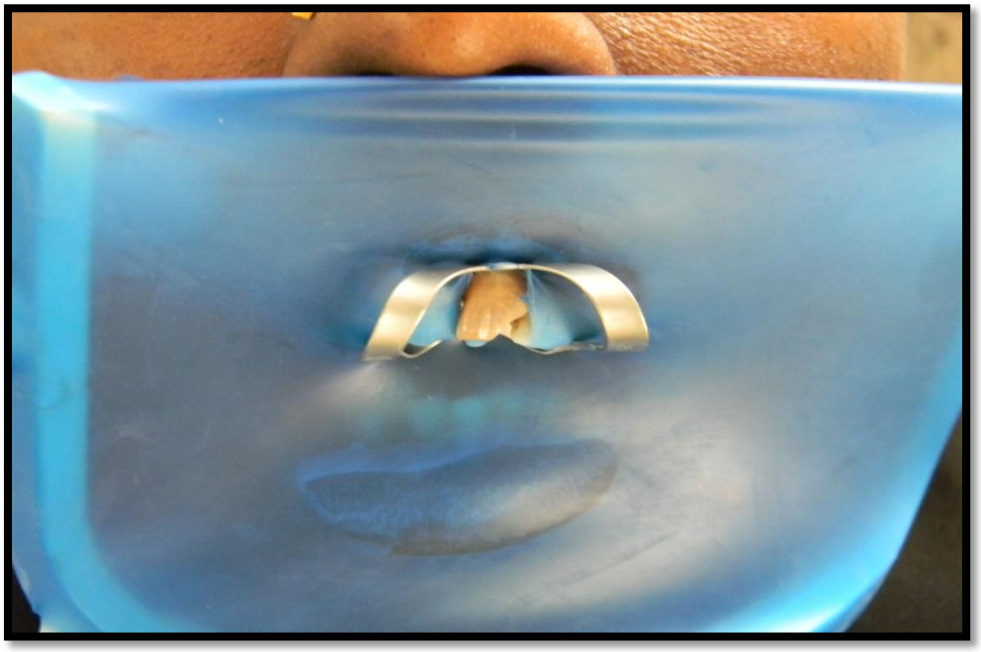


Fig 5 : Rubber Dam Placement(1st Visit)

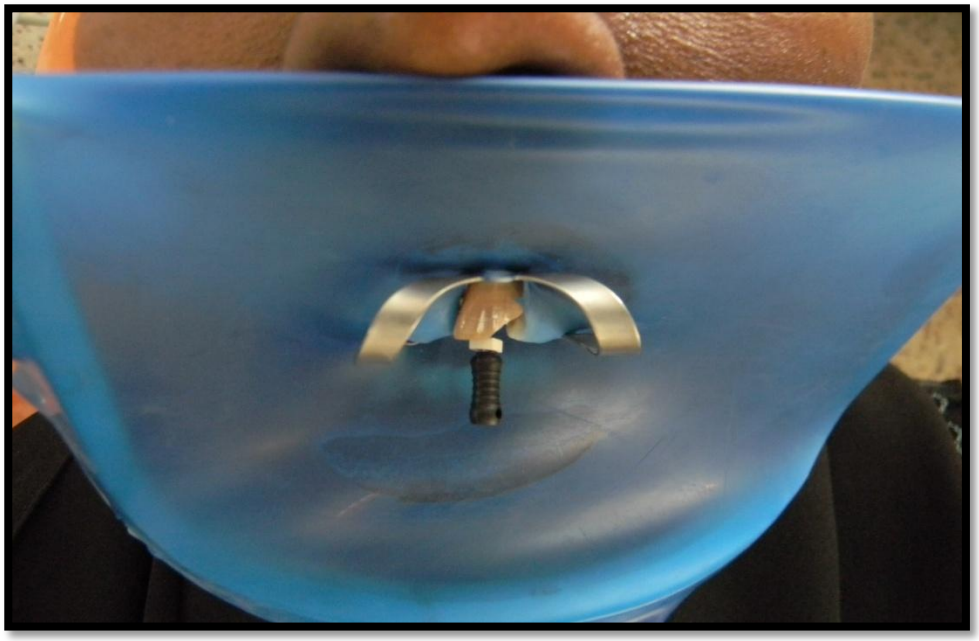


Fig 6: Working Length Determination (1st Visit)

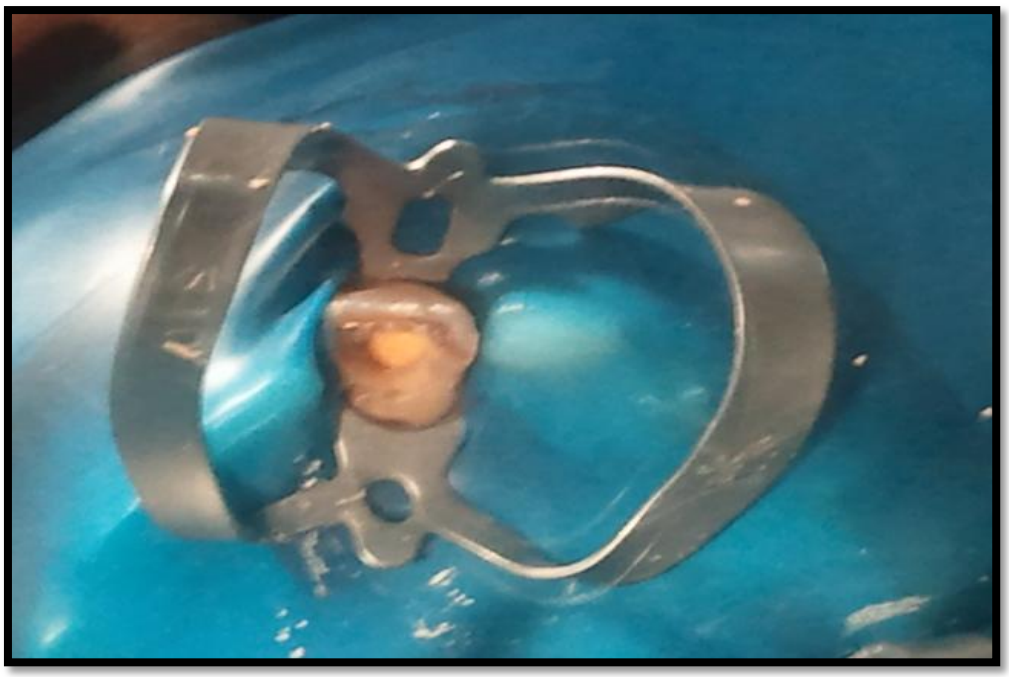


Fig 7: Placement Of Triple Antibiotic Paste (1st Visit)

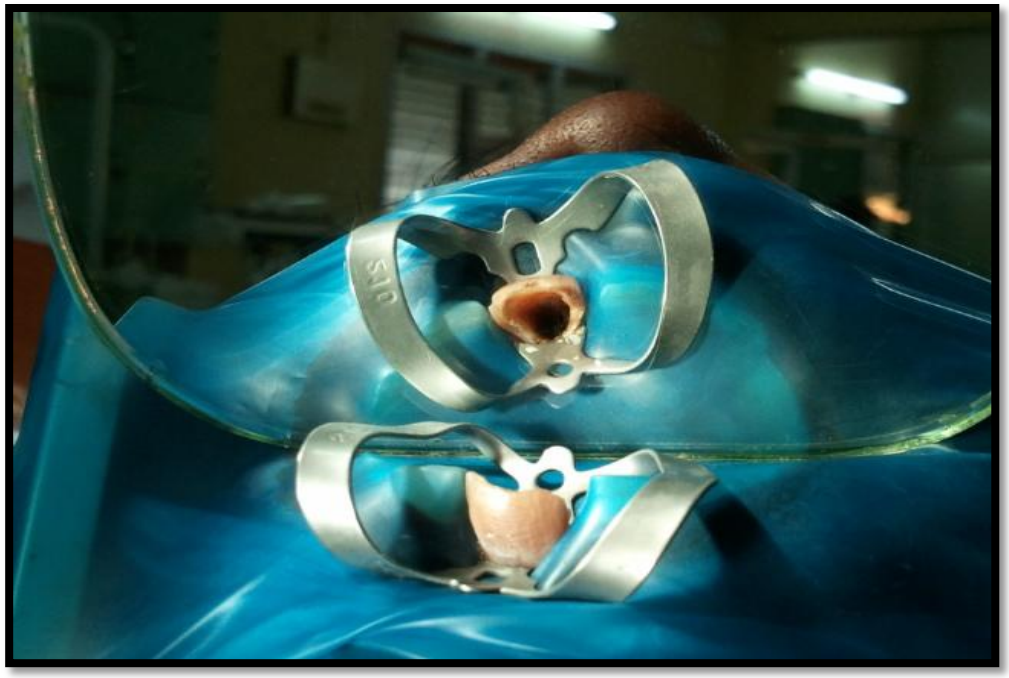


Fig 8 : Reaccessed The Tooth(2nd Visit)

Fig 9: Antibiotic Mixture Washed Out By Irrigation (2nd Visit)

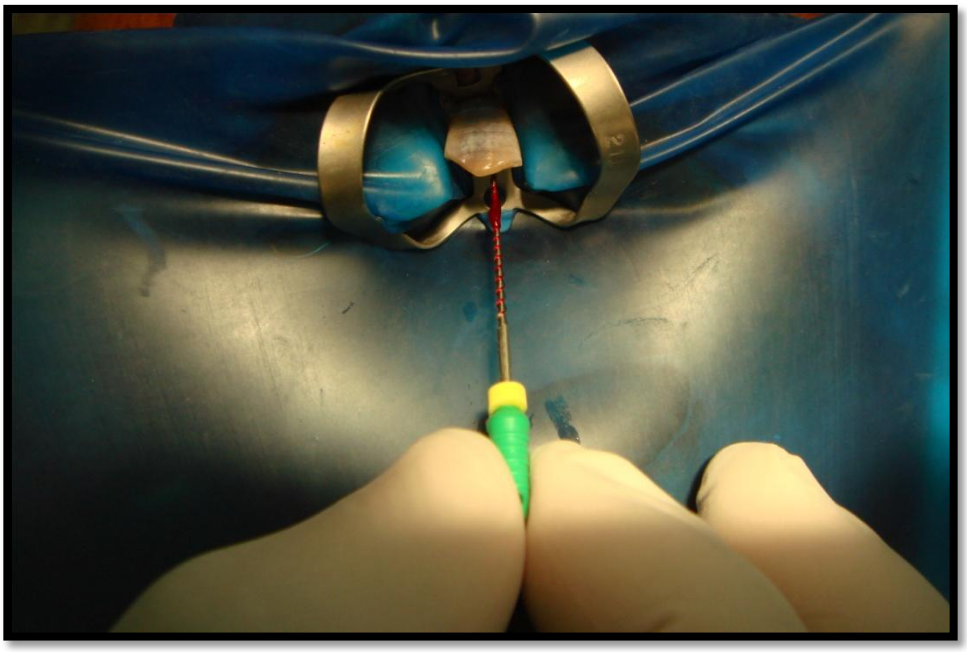


Fig 10: Induce Bleeding By Over Instrumentation(2nd Visit)

Fig 11: MTA Placed At The Leval Of CEJ(2nd Visit)

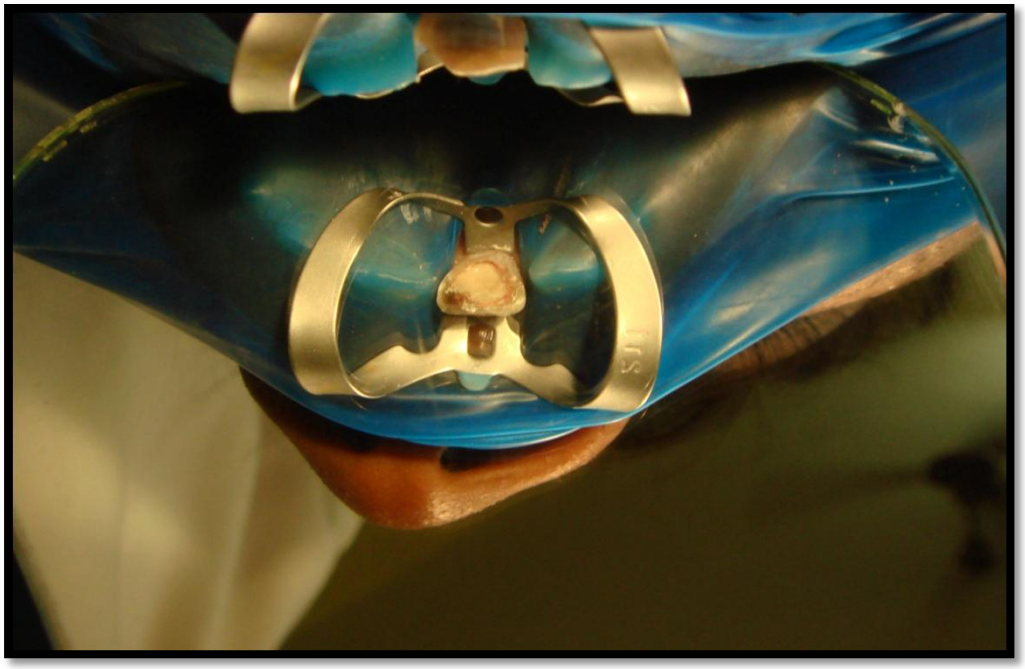


Fig 12 : Access Sealed With Resin Bonded Cement(Double Seal)

RESULT

TABLE 1: EVALUATION OF PULP REVASCULARIZATION

Case no	Gender	Age	Follow up months	Signs and symptoms	Radiographic examination			Electric pulp tester
					Resolution of periapical radioluciences	Apical closure and root lengthening	Evidence of lateral dentin wall thickness	
1	Male	15	10	Absent	+	+	+	-
2	Male	12	10	Absent	+	+	+	-
3	Male	12	10	Absent	+	+	+	+
4	Female	18	10	Absent	+	+	+	-
5	Male	35	Not reported	Absent	NA	NA	NA	NA
6	Male	23	10	Absent	+	-	-	-
7	Female	20	10	Absent	+	+	+	+
8	Female	19	Not reported	Absent	NA	NA	NA	NA
9	Male	16	10	Absent	+	+	-	-
10	Male	32	10	Absent	+	-	-	-
11	Male	23	10	Absent	+	+	+	-
12	Male	26	10	Absent	+	+	+	-
13	Male	29	10	Absent	+	-	-	-
14	Female	24	10	Absent	+	+	-	-
15	Female	24	10	Absent	+	+	+	-

Table 2: INFERENCE TILL 8 MONTHS FOLLOW UP

Features	No. of cases showing positive result			
	2months follow up	4 months follow up	6 months follow up	8 months follow up
Resolution of periapical radiolucencies	15	10	13	13
Increase of Root length	-	-	8	8
Apical closure	-	-	8	8
Increased in width of root dentin	-	-	-	8
Root lengthening and apical closure	-	-	8	8
Response to pulp sensibility test using electric pulp tester	-	-	-	-

TABLE 3: INFERENCE AFTER 10 MONTHS FOLLOW UP

Features	No of cases showing positive result (10 months follow up)
Resolution of periapical radiolucencies	13
Increase of Root length	10
Apical closure	10
Increased in width of root dentin	8
Increase of Root length and apical closure	10
Response to pulp sensibility test using electric pulp tester	2

RESULT

Of the 15 patients who were included in the study, 10 were male and 5 were female. The age, gender, follow up months, sign and symptoms, radiographic evaluation and electric pulp tester response of these cases were tabulated.

Of 15 patients, 2 patients did not report for the follow up after two months. Remaining 13 patients showed no signs and symptoms during the follow up period. 10 patients showed complete resolution of radiolucencies in the periapical region after a follow up period of 10 months. These 10 cases also showed evidences of continuous root lengthening and apical closure. However, increased lateral dentin wall thickness was elicited only in 8 out of these 10 cases after 10 months follow up.

Radiographic finding of the other 3 cases showed resolution of radiolucencies after the sixth month follow up. But there was no evidence of root lengthening or apical closure till ten months follow up.

Out of 13 patients, two patients showed positive response to pulp sensibility test using electric pulp tester.

RADIOGRAPHS

CASE NO :1

Image 1: Pre Operative (WL)

Image 2: After MTA Placement

Image 3: Post Operative(6th Month)

Image 4: Post Operative(10month)

CASE NO :2

Image 5:Pre Operative (WL)

Image 6 : After MTA Placement

Image 7: Post Operative(8th Month)

Image 8 : Post Operative(10th Month)

CASE NO:3

Image 9 : Pre Operative

Image 10: Postoperative (6 Months)

Image 11: Postoperative (10 Months)

CASE NO: 4

Image 12 : Pre Operative

Image 13 : After MTA Placement

Image 14: Postoperative (10 Month)

CASE NO:5

Image 15: Pre Operative (WL)

Image 16: After MTA Placement

Image 17: Post Operative (10month)

CASE NO:6

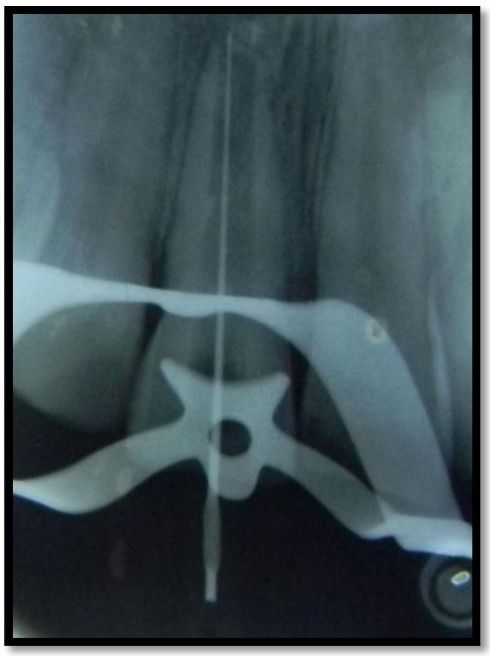


Image 18 : Pre Operative(WL)

Image 19: After MTA Placement

Image 20: Post Operative (10 Month)

CASE NO :7

Image 21: Pre Operative (WL)

Image 22: After MTA Placement

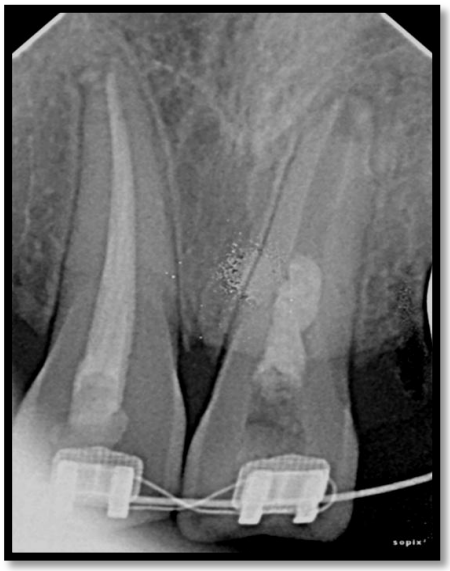


Image 23: Post Operative (10 Months)

CASE NO:8

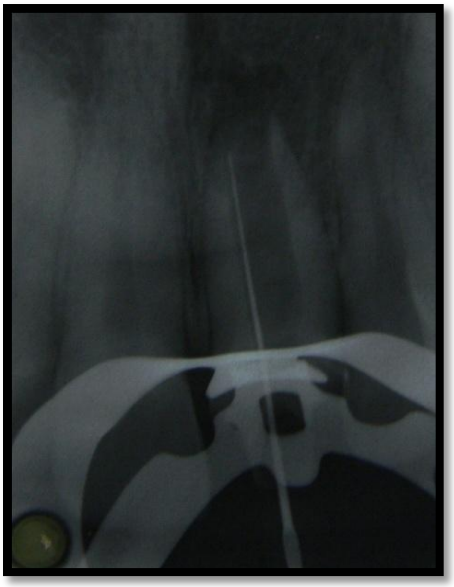


Image 24: Preoperative(WL)

Image 25: After MTA Placement

Image 26: Post Operative

CASE NO :9 AND 10

Image 27:Pre Operative(WL)

Image 28: After MTA Placement

Image 29: post operative

DISCUSSION

Regeneration of tissues rather than conventional treatment options is an emerging and exciting field in the health sciences. Regeneration in endodontics aims to heal infected, nonvital, immature and open apex teeth by regeneration of pulp tissues and apexogenesis. Revascularization is the technique to re-establish the vitality in a previously nonvital tooth and necrosed tooth.[51]

It was earlier thought the periapical tissue of a nonvital infected tooth will not regenerate. Therefore, treatment option followed for such teeth was to perform surgical endodontic procedure to seal the wide-open apex, with retrograde seal. It is an invasive procedure which has its own drawbacks viz discomfort to patient and common complications associated with any surgery. It can also lead to a compromised crown root ratio in a tooth already weakened due to immature root development.[51]

It was Mitchell and Shankhwalkar in 1958 who first documented osteogenic potential of calcium hydroxide. They implantated calcium hydroxide in rat's connective tissue, and reported calcified material was

seen to be deposited, even in an area where no preexisting hard tissue was present[40]. With this study as base, calcium hydroxide apexification in a non vital incisor tooth was documented by Kaiser[31] in 1964 and later popularized by Frank.[16] Since then, apexification has become the conventional standard treatment protocol for treatment of nonvital immature teeth.

However, calcium hydroxide–induced apexification has several disadvantages. (1) First it requires a period of 6 –24 months for barrier formation[1]. (2)The barrier thus formed is often porous and non continuous and therefore requires obturation of the canal after barrier formation, without splitting the tooth[2]. (3) Apexificaiton can only induce a hard tissue barrier at the apex and further development of the root does not take place[2].(4) Calcium hydroxide, physical barrier will block the migration of multipotent undifferentiated mesenchymal cells into the canal and thereby preventing regeneration of tissues at the lateral dentinal walls. (5)By repeated intracanal calcium hydroxide dressing tooth becomes brittle because of the hygroscopic and proteolytic properties of Ca(OH)2 which can lead to fracture.[2] Cvek reported 4 years after calcium hydroxide apexification, fractures ranged from 77% of the

most immature teeth to 28% of the most fully developed teeth[12] . Last but not least, the pH of calcium hydroxide which is known to be toxic to vital cells and hence might damage the cells at the apex, which are presumed to have regenerative capacity.

The emergence of MTA as an artificial apical barrier material has drastically altered the conventional means of management of open apex because of several advantages it poses over calcium hydroxide–induced apexification. MTA is a biocompatible material, has osteoinductive properties, and can even set in the presence of moisture and the entire treatment protocol can be completed in a single sitting.[51]

The major advantage is that the success of treatment by MTA is less dependent on patient co-operation. $Ca(OH)_2$ apexification requires a motivated patient who has to return for multiple follow-up appointments,(the usual recall period for $Ca(OH)_2$ apexification is 3 months until the barrier form) whereas the MTA root-end barrier procedure can be completed in one or two appointments. Allowing a immediate restoration is another advantage of MTA. $Ca(OH)_2$ treatment requires that a patient be left in a provisional restoration for an considerable period of time which is the time required for $Ca(OH)_2$

paste to fulfill its prime function. This intermediate time period makes the tooth vulnerable to crown / root fracture. Andreasen et al showed that $Ca(OH)_{2,}$ intracanal dressing for more than 1 month significantly reduced the fracture resistance of the tooth[23]. Inspite of MTA having numerous advantages over Ca(OH)2, it has its own disadvantages as follows, it lacks the potentiality to induce the thickening of root dentin, it does help in increasing the root length which in turn doesn't improve the crown root ratio.

In concern with above aspects, revitalization procedure promises to overcome the negative aspect of Ca(OH)2 and MTA method of apexification. The important step in revitalization procedure is to disinfect the canal using the right irrigation protocol along with local application of antibiotics.

However, systemic antibiotics have long acted as an adjunct in endodontic therapy. But, they have their own drawbacks such as potential risk of adverse systemic side effects, allergic reactions, toxicity and the rapid development of resistance. For systemic antibiotics to be effective its absorption through the gastro-intestinal tract and distribution via the circulatory system to the infected site is crucial. Hence, the

vitality of a tooth is an important pre- requisite for the action of systemic antibiotics which is not possible in all scenarios. An alternative approach is local application of antibiotics within the root canal system which will be more effective mode for delivering the drug.[42]

The infected root canal system offen shows a polymicrobial microbiota, consisting of both aerobic and anerobic bacteria . Because of the polymicrobial nature of root canal infection it is very much unlikely that a single antibiotic could result in effective disinfection of the canal which necessitates a combined antibiotic regimen[60]. The recommended regimen is metronidazole + ciprofloxacin + minocycline. Sato et al and Hoshino et al.in their two independent studies have showed that this recommened regimen to be very effective in the sterilization of, necrotic pulps, and infected root dentin of permanent teeth [61].

Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. It is a potent bacteriocidal agent. It has been used extensively both systemically and topically in the treatment of periodontal disease. It readily permeates through the bacterial cell membranes and binds to the DNA, disrupting its helical structure, leading to rapid cell death[42].

Ciprofloxacin, a synthetic floroquinolone, is bactericidal in nature. The mechanism of action is by inhibition of DNA gyrase, resulting in disruption of the DNA by exonucleases. Since it is very active against gram-negative organisms when compared to gram positive organism, it is often combined with metronidazole in the treatment of mixed infections[61].

Minocycline is a semisynthetic derivative of tetracycline, are a group of bacteriostatic antimicrobials. They have a broad spectrum of activity against both gram-positive and gram-negative microorganisms. Tetracyclines are effective against most spirochaetes, and many anaerobic and facultative bacteria. The tetracyclines gain access to bacterial cells by passive diffusion through the outer membrane followed by active transport through the inner membrane. They then act by inhibiting protein synthesis on the surfaces of ribosomes[42]. The main drawback of the minocycline is that, it causes discoloration of the tooth.

The non discoloring property of clindamycin has lead to its popularity in replacing minocycline in the recommended antibiotic regimen. Lin et al showed that clindamycin significantly reduced the

amount of viable bacteria in each dentine layer compared with the tetracycline[61].

Clindamycin is a lincosamide antibiotic. It inhibits bacterial protein synthesis by interfering with the transpeptidation reaction, which ultimately retards early chain elongation[61].

The dental pulp is a soft tissue of ectomesenchymal origin, developing from the dental papilla. The compositions of dental pulp are water, ground substance, connective tissue, blood vessels, nerves, lymphatics, fibroblasts, immune cells and odontoblast (Trowbridge 2003). The odontoblasts secrete dentin and are integral to the pulp–dentin complex. Formation of primary dentin take place until root development completes, following which dentin formation proceeds at a slower rate as secondary dentinogenesis. As the root and pulp develop, the dental papilla is located apical to the developing pulp and is called the apical papilla (Tencate 1997).

The compenent of the apical region of an immature permanent tooth consist of dental pulp, apical papilla and periodontal tissues, which arises through a series of ectomesenchymal interactions. During the bell stage of tooth development, the dental papilla becomes partially enclosed

by the invaginating epithelium, and the condensed ectomesenchyme surrounding the enamel organ and dental papilla forming the dental follicle[17].

The dental follicle surrounding the developing root is composed of progenitor cells for the developing periodontium, cementum, alveolar bone and PDL. At the same time, the inner and outer enamel epithelia fuse to form a structure known as Hertwig's epithelial root sheath (HERS). As HERS migrates apically, the ectomesenchymal tissues are divided into the dental papilla on one side and dental follicle on the other. It is argued that HERS has a role in root development and shape, but the exact function of the cells is not completely ascertained[17].

They are believed to be involved in regulating the differentiation of odontoblasts or cementoblasts which are the responsible for formation of dentine and cementum. Once the first layer of mantle dentine has formed, the root sheath begins to disintegrate, allowing the attachment of cells from the dental follicle onto the exposed root dentine with the subsequent deposition of cementum. Individual cells from the root sheath migrate away from the root portion of the tooth to the region of the future periodontal ligament to form the rests of Malassez[17].

HERS is very sensitive to traumatic injury and once destroyed, there is cessation in normal root development with no further odontoblast differentiation. In an immature permanent tooth, this leads to an open root apex with thin weak root walls and a discontinuous periodontal ligament[17].

Under certain condition an open apex can also form in a mature root. Due to extensive apical resorption as a result of orthodontic treatment, inflammatory root resorption and periradicular surgery.

In these scenarios, the ideal treatment option is to obtain further root development and thickening of dentinal walls which can be achieved by regeneration of a functional pulp-dentin complex using revascularization procedures.

Revascularization is the procedure to reestablish the vitality in a nonvital tooth to allow repair and regeneration of tissues. The rationale of revascularization is to create a sterile environment with proper coronal seal in which new cells can grow in the tissue matrix leading to the establishment of of pulp like tissue[6].

The aim of this study was to evaluate the revascularization procedure both in immature and mature permanent teeth with necrotic pulp and open apices, disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

The study was approved by the Ethical committee of Ragas dental college and hospital and due clearance was obtained for carrying out the investigation. A total number of 15 cases were selected from those patients who were referred to the Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital for root canal therapy. An informed consent was signed by all the patients participating in the study and were clearly informed about the duration of study period which was 10 months.

Patients in the age group between 07 and 35 years who were immunocompetant and do not have any systemic diseases were selected for the study. Teeth with open apex that were verified using preoperative radiographs only were selected. Teeth having root fractures and non restorable crown all of which were checked using radiographs were excluded from the study.

All teeth were treated by a common, regenerative endodontic protocol. On the first visit, the pulp chamber was accessed after local anesthesia with adrenaline and isolated using rubber dam. The working length was determined radiographically by inserting #15 size K-file into the canal at the estimated preoperative canal length. Each root canal orifice was gently irrigated with 10 mL of 3% NaOCl without any instrumentation. The canals were then dried with paper points. The triple antibiotic paste used in this study consisted of freshly mixed antibiotic powders of metronidazole, ciprofloxacin, and clindamycin in 1:1:1 ratio. They were mixed with distilled sterile water to form a paste-like consistency. This antibiotic paste was then packed into canal using a sterile Lentulo spiral file till the level of cemento-enamel junction. The access was then cleaned with sterile cotton pellets followed by the placement of IRM for the coronal seal. The antibiotics paste was left in the canal for a period of two weeks.

In the second visit, all teeth were asymptomatic and showed radiographic evidence of reduction in periapical radiolucency. The teeth were anesthetized with 2% lignocaine without a vasoconstrictor, (presence of vasoconstrictor will not aid in

inducing apical bleeding since it decreases blood flow in this region) isolated with a rubber dam, and reaccessed. The antibiotic mixture was washed out by using sterile saline irrigation. The canal was dried with paper points. The apical tissue beyond the confines of the root canal were stimulated with a sterile H-file (35-50) to induce bleeding into the canal space. Approximately 15 minutes was allowed for blood clot to reach the level approximately 2–3 mm below the cemento-enamel junction. A blood clot was produced to the level of the cement-enamel junction to provide a scaffold, followed by a double seal of MTA in the cervical area and a bonded resin coronal restoration over it. Patients were recalled for every 2 months interval in the period of 10 months. In this follow up, patients were subjected to clinical examination, radiographic examination and pulp sensibility test for the evaluation of signs and symptoms, root development and pulp sensibility.

Radiograph should be taken in the method of paralleling technique. Its basis lies in the principle that image sharpness is primarily affected by focal-film distance (distance from the focal

spot within the tube head and the film), object film distance, motion, and the effective size of the focal spot of the x-ray tube. Paralleling technique named because the object (tooth), receptor (film packet), and end of the position indicating device (PID) are all kept on parallel planes.

The common techniques used in practice to assess the pulp status of teeth are Electric pulp testing and thermal tests. Electric pulp testing is based on stimulation of sensory nerves, and requires and relies on subjective assessments and comments from the patient. EPT is more accurate test to check the pulp sensibility. In this present study, EPT was used to check the pulp sensibility[37].

In the second month follow up, all the 15 cases showed no signs and symptoms with a reduction in the periapical radioluciences.

In the fourth month of follow up, 2 cases failed ro report. The remaining 10 out of 15 cases, were asymptomatic with a complete resolution of periapical radioluciences. 3 out of 15 cases were

asymptomatic and only minimal reduction in the periapical radiolucieny was observed radiographically.

In the sixth month follow up, complete resolution of periapical radioluciences were seen in all the 13 cases reported and there was evidence of root lengthening and apical closure in 8 cases. The remaining 5 cases showed showed no evidence of root lengthening or apical closure.

In the eighth month follow up all the 13 cases reported, continued to show complete resolution of periapical radiolucency. 8 among the 13 cases showed root lengthening and apical closure with evidence of lateral wall thickness in root dentin. 2 cases showed satisfied levels of root lengthening and apical closure with no evidence of any lateral thickness. The remaining 3 cases showed no improvements compared with the previous follow ups. Pulp sensibility test was done in this follow up using electric pulp tester. In this follow up, there was no response to electric pulp testing.

In the tenth month follow up, 10 cases showed evidences of root lengthening and apical closure. Out of which there was

evidences of lateral wall thickness in radicular dentin only in 8 cases. Out of these 8 cases that showed evidences of lateral wall thickness, 2 cases responded to electric pulp testing showing pulp sensibility. The remaining 3 out of 13 cases that were reported, continued to show no improvements.

The results of the present study indicated that it is possible to treat both immature permanent tooth and matured permanent open apex tooth which could render the patient asymptomatic without any evidence of sinus tract and resolving apical periodontitis. There is also radiographic evidence of continuing thickness of dentinal walls, apical closure, or further development of root length[29].

In the present study, 2 out of 13 teeth receiving the full revascularization treatment ultimately demonstrated a return of pulp sensibility which is in accordance with the study by Petersson et al showing that teeth with necrotic pulps might give a positive response for the electrical test. However, histological evaluation is the gold standard to demonstrate the regeneration of pulpal tissues in these teeth, but this might not be possible being a clinical study.

The 3 cases showing an unfavorable outcome in the present study might be related to a failure to induce any bleeding into the canal. The absence of a blood clot has been shown to have a negative impact on successful revascularization of the pulp in an animal study. A possible reason might be the resolution of inflammatory reaction after intra canal dressing with the antibiotic paste, making it more difficult to induce bleeding.

Chen MY, Chen KL reported five different types of responses to revascularization procedures in permanent immature teeth with infected necrotic pulp.[9]

Types	Different types of responses to revascularization procedures (Chen MY, Chen KL et al)
Type -I	Increased thickening of the canal walls and continued root maturation
Type -II	No significant continuation of root development with the root apex becoming blunt and closed.
Type -III	Continued root development with the apical foramen remaining open
Type -IV	Severe calcification (obliteration) of the canal space
Type -V	A hard tissue barrier formed in the canal between the coronal MTA plug and the root apex

In accordance to above, 10 out of 13 cases reported a follow up period of 10 months can be classified as type I in the present study.

The possibility for the favourable treatment outcome of the present study, the first possible explanation may be due to the fact that some a few vital pulp cells would have remained at the apical end of the root canal. These cells must have proliferated into the newly formed matrix and differentiate into odontoblasts under the organizational influence of cells of Hertwig's epithelial root sheath, which are highly resistant to disintegration, even in the presence of inflammation . The newly formed odontoblasts can lay down atubular dentin at the apical end, causing apexogenesis.[65]

The second possible explanation of continued root development could be due to multipotent dental pulp stem cells, which can differentiate into odontoblasts and deposit tertiary or atubular dentin leading to elongation of root.[20]

The third possible explanation could be attributed to the presence of stem cells in the periodontal ligament, which can deposit hard tissue at the apex and on the lateral root walls. The presence of cementum and

Sharpey's fibers in the newly formed tissues is being presented as a proof for this explanation.[36]

The fourth possible explanation is attributed to transplantation of stem cells from the apical papilla or the bone marrow to the canal lumen during instrumentation beyond the apex to induce bleeding [33].Fifth possible explanation could be the blood clot itself, which is rich in growth factors. These growth factors include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor(PDEF), and tissue growth factor(TGF) that have the capacity to stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, cementoblasts.[60]

However, this study needs further follow up period of one year to demonstrate progresses in pulp revascularization and to check the responses of the teeth to electric pulp sensibility testing.

The 3 immatured teeth showed increased in root length, increased in thickening of root dentin, apical closure along with resolution of periapical radiolucency which was remarkable proving successful revascularization procedure. The remaining matured tooth showed increase in root length along with apical closure.

The present study evaluated the revascularization procedure by inducing bleeding in the apical tissue. Future studies should be focused by using platelet rich plasma (PRP), platelet rich fibrin (PRF) and comparing them to assess the best procedure that could be followed to yield higher success rates in revascularization procedure.

SUMMARY

This study was done to evaluate the revascularization procedure both in immature permanent teeth with infected pulp and mature permanent teeth with open apices disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

15 patients referred to department of conservative dentistry and endodontics diagnosed with both open apex in immature permanent teeth and matured permanent teeth were selected for the study. The root canal of the teeth were accessed and disinfected using triple antibiotic paste containing ciprofloxacin, metronidazole and clindamycin in the ratio1:1:1. Following the absence of signs and symptoms, blood clot was induced in the root canal. Finally the access was closed using a double with MTA placed apical to cementoenamel junction and resin bonded cement over the MTA.

Revascularization was assessed by evaluating the root development, apical closure, and lateral wall thickening of dentinal walls by radiograph and pulp sensibility by electric pulp tester for an entire duration of the study (10 months) with an interval of 2months between each follow ups.

CONCLUSION

Within the limitations of the present study, the following were observed in immature and mature permanent tooth.

1. All the patients who reported till the 10 months follow up showed complete resolution of periapical radiolucencies.
2. 10 Out of 13 patients, who reported till a period of 10 months follow up showed root development and apical closure.
3. 8 out of 13 patients who reported till a period 10 months follow up showed root development, apical closure and lateral thickening of radicular dentin.
4. 2 out of 13 patients at the end of 10 month follow up showed positive response to electric sensibility test.

All the patients are to be followed up further for a period of 1 year to verify the pulp sensibility.

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